



Body weight loss of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) during development in non-feeding stages: Implications for egg clutch parameters

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Abstract. The developmental stages of the black soldier fly, *Hermetia illucens*, are widely studied because there are several ways in which this species can be utilized. In this context, the aim of the current study is to determine the loss of weight of larvae of different weights between the prepupal and reproductive adult stages and their effect on the egg clutch quality parameters. The *Hermetia illucens* prepupae were categorized in 3 body weight classes. The weight loss was evaluated by weighing individuals in the prepupal, pupal and adult stages. The weight and length of the individuals were positively correlated in the pupal stage and adult females. There were significant losses of weight ($P = 0.000$) in the prepupal, pupal and adult stages in all three weight classes. In each weight class the relationship between weights was significant ($P = 0.000$) for all development stages. The results clearly indicate that the largest larvae of *Hermetia illucens* produced the adults with the best clutch quality parameters and the existence of significant positive correlations between body weight and both weight loss and the egg clutch quality parameters.

INTRODUCTION

Hermetia illucens L. (Diptera: Stratiomyidae) is a species of fly used as a sustainable alternative for managing organic (Čičková et al., 2015; Pastor et al., 2015; Cheng et al., 2017) or municipal waste (Diener et al., 2011). Because of its ability to digest organic matter from different sources, like kitchen waste (Nguyen et al., 2015; Pastor et al., 2015; Salomone et al., 2017), sewage sludge (Lalander et al., 2013; Cai et al., 2017), human faeces (Banks et al., 2014), vegetable waste (Parra Paz et al., 2015) and manure (Myers et al., 2008; Oonincx et al., 2015) it can efficiently reduce the volume of organic matter and produce a valuable fertilizer for agriculture (Choi et al., 2009; Sarpong et al., 2019). Recent studies reveal that the prepupae of the black soldier fly can also be used to produce biodiesel (Zheng et al., 2012; Surendra et al., 2016).

In addition, the ability of this insect to reduce the microbiological contamination of waste has been demonstrated (Liu et al., 2008; Lalander et al., 2015). Other studies show its potential for decontaminating organic substrates (Bulak et al., 2018) contaminated with different heavy metals like Cd (Biancarosa et al., 2017; Gao et al., 2017), Pb and Zn

(Diener et al., 2015) as its larvae accumulate and concentrate the contaminants (Van der Fels-Klerx et al., 2016).

Hermetia illucens larvae are also used as an alternative high protein source for incorporating in feed for farm animals, such as poultry (Kawasaki et al., 2019), swine (Yu et al., 2019), rabbits (Martins et al., 2018; Gasco et al., 2019) and especially aquaculture (St-Hilaire et al., 2007; Cummins et al., 2017; Bava et al., 2019). Moreover, analyses of the biological value of the protein (Makkar et al., 2014; Barragan-Fonseca et al., 2017) and fat in the larvae of *H. illucens* (Kroeckel et al., 2012; Wang & Shelomi, 2017) indicate that the nutritional value of the feed made from the larvae is comparable to fish (Sealey et al., 2011; Rumpold et al., 2018) and soybean meal (Secci et al., 2018; Cullere et al., 2019). The nutritional value of the larvae, however, is directly dependent on their feeding medium (Sprangers et al., 2016; Meneguz et al., 2018; Danieli et al., 2019).

Because of the potential of this species for use in waste recycling and decontamination of environments, and in providing an alternative protein source for farm animals, there are many studies on its biology and ecology (Zhang et al., 2010; Holmes et al., 2012; Nguyen et al., 2013) and

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physiological particularities (Bonelli et al., 2019; Bruno et al., 2019). This research indicates there are concerns about the development and reproduction of this species (Heussler et al., 2018; Wynants et al., 2018; Gligorescu et al., 2019). In particular, the effect of the cessation of feeding at the prepupal stage (Gobbi et al., 2013), which means the reproductive effort is sustained entirely by energy reserves stored during the larval stage (Lupi et al., 2019), which can significantly affect the reproduction of this species. Tomberlin et al. (2009) demonstrate that the mean body weight loss from the prepupal to the adult stage depends on sex and rearing temperature. At 27°C, the body weight loss is 52.5% for males and 49.4% for females and at 30°C it is 50.8% for males and 48.6% for females.

The aim of this study is to determine the weight loss of *H. illucens* from the prepupal stage to the reproductive adult and evaluate the effect of this weight loss on reproduction, in particular weight of each egg clutches, number of eggs in a clutch and egg weight.

MATERIAL AND METHODS

Laboratory study

This study was carried out in the Laboratory of Ecology, Environmental Protection and Zoology at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (Romania). There were three replicates of the experiment carried out from December 2018 to June 2019.

Biological material

The *H. illucens* population originates from a colony obtained and maintained all year round in the aforementioned laboratory. The initial biological material was commercially available pupae from Greece. The colony was kept under appropriate conditions for this species, a temperature $27 \pm 0.2^\circ\text{C}$ and 60% relative humidity (RH) (Tomberlin et al., 2009; Hoc et al., 2019), and a photoperiod of 16 h of yellow and white LED light (Nakamura et al., 2016). For the *H. illucens* population used in this study 15 egg clutches laid within 24 h were collected from the colony. The larvae hatched after 48–72 h and were reared on an organic substrate (containing 13% crude protein), consisting of vegetal waste (cabbage, lettuce, carrots, cucumbers, orange peel), bakery leftovers and kitchen waste. The moisture content of this substrate was 70% and the temperature was between 26 and 27°C (Tomberlin et al., 2002). According to the literature, larvae reared on this substrate are relatively big and healthy (Nguyen et al., 2013).

Experimental design

The larvae were reared in a dark plastic container ($50 \times 34 \times 14$ cm), which contained approximately 2.5 larvae/cm² with 1–1.5 g of substrate/individual (Sheppard et al., 2002). The feeding took place until 40% of the prepupae had dispersed. When the larvae were 23–25 days old they were in the prepupal stage, ceased feeding and left the substrate. Over a 24 h period the migrating prepupae were collected, sorted into weight classes and placed on a dry medium of wood shavings (< 20% moisture) in three plastic containers ($15 \times 10 \times 5$ cm) with ventilation holes and kept under the standard laboratory conditions of 27°C and 60% RH until the adults emerged. The black soldier flies were kept in rearing cages ($50 \times 35 \times 45$ cm) under standard laboratory conditions of $27 \pm 0.2^\circ\text{C}$ and 60–70% RH (Park et al., 2010; Nakamura et al., 2016). A plastic box ($20 \times 15 \times 10$ cm) was placed in each cage, which contained an attractive substrate for oviposition. This was cov-

ered with mesh and a surface for oviposition placed on top of it, which consisted of 10 wooden plates with gaps of 4 mm between them (Boaru et al., 2019). The wooden structure was replaced every day with a new, sterile one.

The prepupae that migrated from the feeding medium within 24 h were weighed individually and grouped according their body weight into 3 weight classes: 150–200 mg (W_1); 200–250 mg (W_2) and 250–300 mg (W_3).

Parameters measured

The following were observed and recorded for *H. illucens*: (1) the loss in weight in each weight class during the prepupal, pupal and adult stages; (2) the influence of body weight on the weight of an egg clutch, number of eggs in a clutch and mean egg weight; and (3) on the sex ratio. Furthermore, the correlations between body weight and weight loss, weight of an egg clutch, number of eggs in a clutch and mean weight of males and females were determined.

Every 3 days, the body weights of the prepupae were determined by weighing, until they pupated and that of the pupae until the adults emerged. The body weight of the adults was recorded after death. The weight loss in the prepupal stage was evaluated every 3 days (E_1 to E_6) for the individuals in each weight class.

At the beginning of the pupal stage, the weight and length of each pupa was determined. As pupation occurred on different days the weight loss of individuals was recorded for recent pupations at regular intervals (E_1 to E_3). The pupation period is the time from recording the first pupa until pupation ceased. The weight loss of the pupae was measured by weighing the individuals in each weight class every 3 d until the flies emerged (E_1 to E_3). Also, after the flies emerged the exuviae were weighed for the individuals in each weight class.

For the adults, the onset of mating and oviposition, the number of egg clutches, the weight of a clutch of eggs, number of eggs in each clutch and mean egg weight for each clutch were recorded. The association between body weight and sex ratio was determined after the adults died and were individually sexed, weighed and their length measured.

An electronic balance (Explorer-Pro model EP114C, precision 0.01 mg) and electronic callipers (model Stainless Precise 7215) were used for weighing and measuring. The counting of the eggs was carried out under a binocular microscope, model Alpha (zoom 7×–45×). The eggs in a clutch were separated using 99% alcohol.

Statistical analyses

Data were analysed using Microsoft Excel 2010. An ANOVA was used to test the differences between groups followed by one-way ANOVA using Student t-test as a post-hoc test for comparison, with a 5% significance level. For interpreting the differences in the weights of the clutches of eggs and numbers of eggs, a Mann-Whitney test was used along with a 1% significance level. The Pearson correlation coefficient (r) was determined and compared for 3 significance levels (5%, 1%, 0.1%). Data are presented as means \pm standard error ($\bar{X} \pm \text{SEM}$). Figures were created using Microsoft Excel 2010.

RESULTS

The body weights of the prepupae leaving the substrate increased for all weight classes with the first to leave being lighter than those that left last Fig. 1. After the prepupae of the different weight classes left the substrate, 100 W_1 individuals (N), 200 W_2 individuals and 100 W_3 individuals were collected.

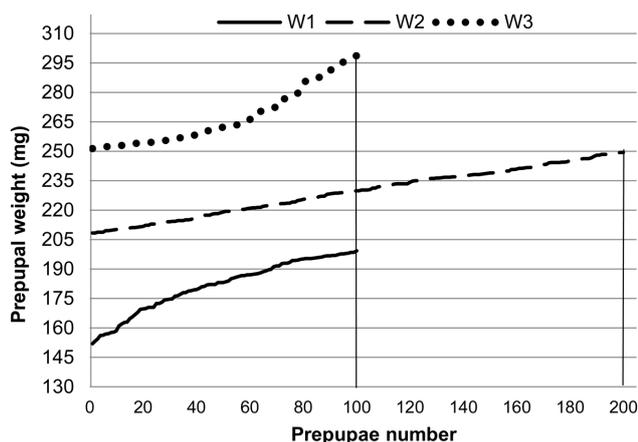


Fig. 1. Trends in the weights of the prepupae leaving the substrate recorded for the three different weight classes.

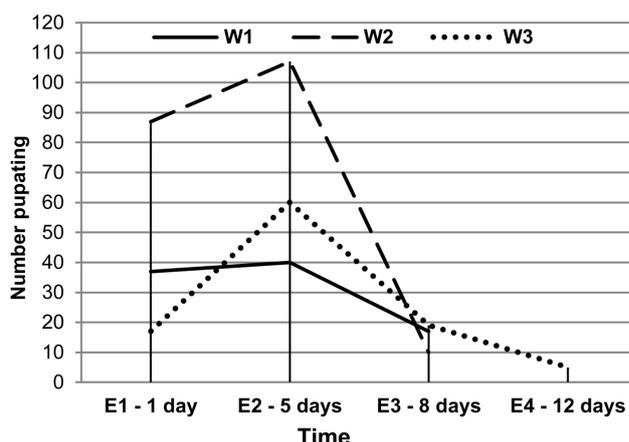


Fig. 3. Numbers pupating recorded at 5 day intervals for each of the different weight classes.

The body weight evolution in the prepupal stage

The duration of the prepupal stage was 21 ± 1 d in all three weight classes. Thirteen individuals in weight class W_1 , 3 in W_2 and 2 in W_3 did not pupate.

The decrease in body weight in the prepupal stage was different in the three weight classes (Fig. 2A and B). For W_1 the mean initial body weight of prepupae was 181.61 ± 1.3 mg and on the last day (E_6) it was 158.46 ± 3.4 . For W_2 it was 229.26 ± 0.9 mg and 185.97 ± 12.9 mg, respectively, and for W_3 it was 267.51 ± 1.5 mg and 212.50 ± 30.5 mg, respectively (Fig. 2A and B).

Over the period analysed, weight losses differed in the three weight classes. For W_1 significant weight losses were recorded ($F = 17.06$; $df = 5$; $P = 0.000$). Over the first 10 days there was a significant weight loss ($p < 0.001$) and then it remained constant ($p > 0.05$). For W_2 significant weight losses were recorded ($F = 69.33$; $df = 5$; $P = 0.000$) and for 16 days the weight losses recorded for the prepupae were significant ($p < 0.001$) after which the loss was insignificant ($p > 0.05$). For W_3 , the weight loss was significant ($F = 29.99$; $df = 5$; $P = 0.000$) and similar to that recorded for W_2 .

There were significant differences between the mean body weight of the prepupae in the different weight classes throughout this stage ($F = 1981.46$; $df = 2$; $P = 0.000$).

Body weight in the pupal stage

The pupal stage lasted 14 to 16 days in all weight classes. Not all individuals pupated on the same day, thus pupation lasted 8 days in W_1 and W_2 , and 12 days in W_3 . The peak pupation number was recorded after 5 days for all weight classes (Fig. 3).

The body weights ($F = 10.46$; $df = 2$; $P = 0.000$) and lengths ($F = 6.95$; $df = 2$; $P = 0.002$) of the W_1 individuals that were the last to pupate were significantly lower than that of those that pupated earlier (Table 1). For the W_2 individuals there were also significant differences in weights of the pupae ($F = 7.79$; $df = 2$; $P = 0.001$) with those that pupated first (E_1) heavier than those that pupated last (E_3). No significant differences ($F = 1.50$; $df = 2$; $P = 0.225$) were recorded in the lengths of the pupae in relation to the pupation period. For W_3 there are no significant differences in the weights ($F = 0.335$; $df = 3$; $P = 0.800$) and lengths of the pupae ($F = 1.30$; $df = 3$; $P = 0.280$) recorded on different days (Table 1).

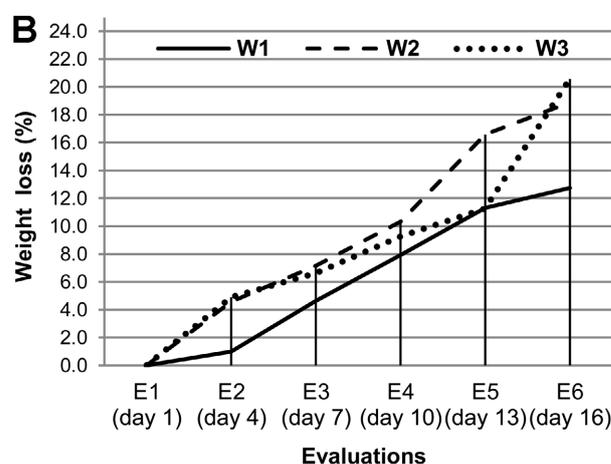
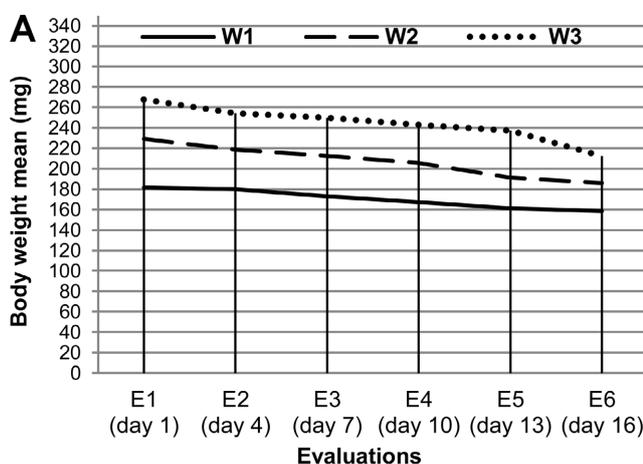


Fig. 2. Decrease in body weight (A) and percentage weight loss (B) of prepupae recorded at 3 day intervals for each of the different weight classes.

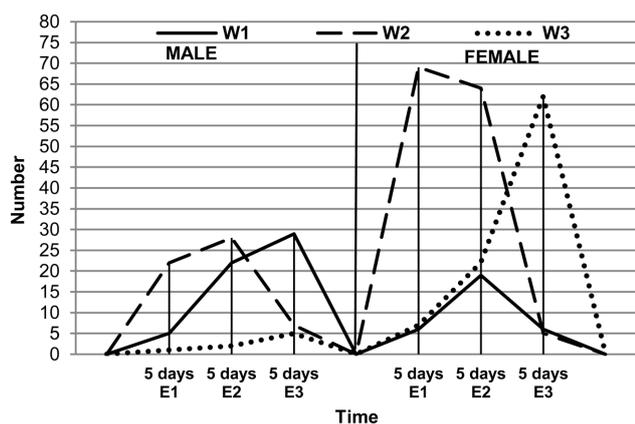


Fig. 4. Numbers of males and females that were recorded dead every five days post emergence for the different weight classes.

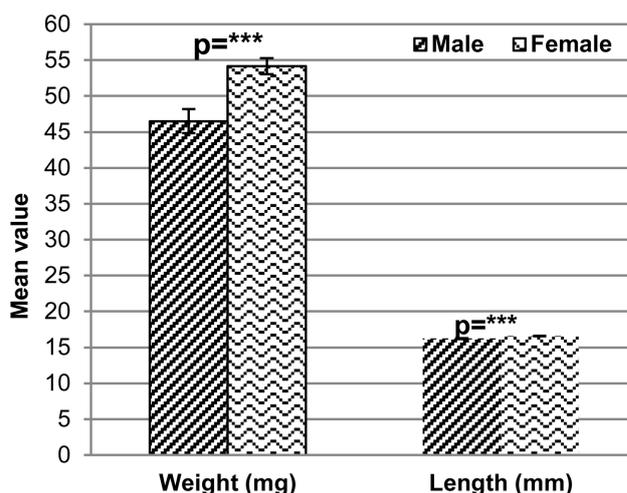


Fig. 5. The weights and lengths of the males and females.

There were significant differences between the different weight classes in body weight ($F = 562.25$; $df = 2$; $P = 0.000$) and length ($F = 188.65$; $df = 2$; $P = 0.000$) of the individuals at pupation. The mean body weight and length at pupation of W_1 individuals was 166.96 ± 1.7 mg and 21.16 ± 0.1 mm, respectively. For W_2 it was 207.22 ± 0.9 mg and 22.76 ± 0.1 mm, respectively. For W_3 it was 240.9 ± 1.8 mg and 24.04 ± 0.1 mm, respectively.

There were very significant correlations ($p < 0.001$) between the mean weight and mean length of the pupae in each weight class: for W_1 $r = 0.689$; W_2 $r = 0.338$; W_3 $r = 0.466$.

There were significant weight losses in the pupal stage in each weight class (Table 1), but the pupal development period was 15 d in each weight class ($p > 0.05$). For W_1 and W_2 the pupal weight decreased significantly ($F = 33.94$; $df = 4$; $P = 0.000$ and $F = 59.38$; $df = 4$; $P = 0.000$) from E_1 to E_5 . For W_3 there was a significant weight loss over time ($F = 9.88$; $df = 4$; $P = 0.000$) starting from E_2 (Table 1). There were significant differences in the body weights of the different weight classes ($F = 1305.41$; $df = 2$; $P = 0.000$).

The mean weight of the exuviae for W_1 individuals was 29.37 ± 0.4 mg, for W_2 36.74 ± 0.5 mg and for W_3 45.70 ± 0.9 mg. The mean weight of only the exuviae of W_3 individuals that emerged last were significant different from that of those that emerged earlier ($F = 17.47$; $df = 2$; $P = 0.000$). The differences were not significant for W_1 ($F = 0.70$; $df = 3$; $P = 0.553$) and W_2 ($F = 1.56$; $df = 2$; $P = 0.214$). There were significant differences in the weights of the exuviae recorded for the different weight classes ($F = 128.24$; $df = 2$; $P = 0.000$), which were correlated with the mean body weights of the different weight classes.

Body weight and mortality in the adult stage

Two individuals in the W_1 and W_2 , and one in the W_3 weight classes did not emerge. The length of life of the adults was 10–15 days for W_1 individuals, 7–11 days for W_2 and 8–11 days for W_3 .

The number of adult males in the W_1 weight class that died peaked at 14–15 days (E_3) and that of females at 10–11 days (E_2) (Fig. 4).

Table 1. Changes recorded in pupal traits at pupation and during pupation. Different letters in a column indicate significant differences at 5% level using t-test as the post hoc test. E – evaluations; S^2 – variance.

Lots	E	Pupal weight (mg)						Pupal length (mm)		
		At pupation			During pupal phase			At pupation		
		S^2	$\bar{X} \pm SEM$	P-value	S^2	$\bar{X} \pm SEM$	P-value	S^2	$\bar{X} \pm SEM$	P-value
W_1	E_1	206.7	174.25 ± 2.4^a	0.006	206.7	174.25 ± 2.4^a	0.006	0.8	21.59 ± 0.1^a	0.010
	E_2	145.2	165.66 ± 1.9^b	0.057	165.3	166.30 ± 1.5^b	0.000	0.6	21.09 ± 0.1^b	0.167
	E_3	491.9	154.15 ± 5.4^b	0.002	218.4	156.61 ± 1.6^c	0.000	4.0	20.37 ± 0.5^b	0.026
	E_4	–	–	–	353.3	142.65 ± 3.1^d	0.000	–	–	–
	E_5	–	–	–	914.5	$122.84 \pm 11.4^{e,d}$	0.003	–	–	–
W_2	E_1	159.6	210.52 ± 1.4^a	0.007	159.6	210.52 ± 1.4^a	0.000	0.9	22.90 ± 0.1^a	0.055
	E_2	148.5	205.66 ± 1.2^b	0.185	154.5	204.36 ± 0.9^b	0.000	0.8	22.65 ± 0.1^a	0.943
	E_3	512.4	$195.25 \pm 7.2^{a,b}$	0.062	149.7	194.44 ± 0.9^c	0.000	1.4	22.71 ± 0.4^a	0.802
	E_4	–	–	–	159.8	187.04 ± 1.4^d	0.000	–	–	–
	E_5	–	–	–	183.5	$177.60 \pm 4.8^{e,d}$	0.000	–	–	–
W_3	E_1	122.0	241.55 ± 2.7^a	0.907	122.0	241.55 ± 2.7^a	0.620	1.1	24.85 ± 0.3^a	0.606
	E_2	348.8	239.97 ± 2.4^a	0.378	316.9	239.87 ± 2.0^a	0.028	0.8	24.39 ± 0.1^a	0.104
	E_3	377.8	237.44 ± 4.5^a	0.436	336.9	233.77 ± 1.9^b	0.020	0.8	24.00 ± 0.2^a	0.109
	E_4	426.2	235.00 ± 9.2^a	0.802	330.8	224.94 ± 1.9^c	0.000	0.3	23.89 ± 0.2^a	0.923
	E_5	–	–	–	299.6	$226.86 \pm 2.7^{d,c}$	0.000	–	–	–

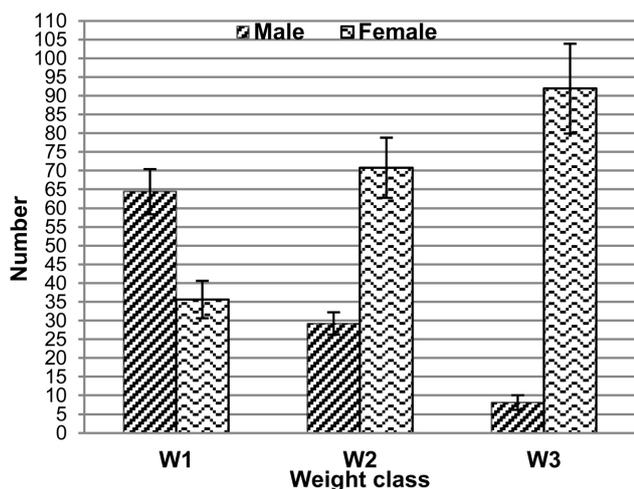


Fig. 6. Numbers of males and females recorded emerging in the different weight class treatments.

For W_2 individuals the peak in the number of males dying was recorded at 9–10 days (E_2) and that of females at 5–7 days (E_1). For W_3 the peaks in the numbers of males and females dying was recorded at 14–15 days (Fig. 4).

During the adult stage there were significant body weight losses in all weight classes (Table 2). For W_1 individuals there were significant weight losses recorded for both males ($F = 20.03$; $df = 2$; $P = 0.000$) and females ($F = 13.23$; $df = 2$; $P = 0.000$). However, a higher loss was recorded for females (Table 2). The length of the flies did not differ significantly between those that died early and late for males ($F = 0.32$; $df = 2$; $P = 0.729$) or females ($F = 2.78$; $df = 2$; $P = 0.079$). For W_2 individuals the weight at E_1 was significantly different from that at E_3 and relatively similar for both sexes: males ($F = 10.01$; $df = 2$; $P = 0.000$) and females ($F = 27.00$; $df = 2$; $P = 0.000$). Also, in terms of length the differences between E_1 and E_3 , for both sexes: males ($F = 0.60$; $df = 2$; $P = 0.552$) and females ($F = 2.50$;

Table 2. Changes in weight and length during the adult stage. Different letters in a column indicate significant intra-sex differences, at a 5% level using t-tests as the post hoc test. E – evaluations; S^2 – variance.

Weight classes	Sex	E	Adult weight (mg)			Adult length (mm)		
			S^2	$\bar{X} \pm SEM$	P-value	S^2	$\bar{X} \pm SEM$	P-value
W_1	M	E_1	594.1	64.12 ± 10.9 ^{a,b}	0.087	0.4	15.60 ± 0.2 ^a	0.301
		E_2	54.6	39.25 ± 1.6 ^a	0.030	0.8	15.85 ± 0.2 ^a	0.603
		E_3	49.0	34.67 ± 1.3 ^b	0.055	0.7	15.74 ± 0.1 ^a	0.501
	F	E_1	472.3	62.85 ± 8.9 ^a	0.034	0.5	15.77 ± 0.3 ^a	0.375
		E_2	119.1	37.28 ± 2.5 ^b	0.027	0.2	15.47 ± 0.1 ^a	0.168
		E_3	32.2	28.15 ± 2.3 ^c	0.009	0.4	15.05 ± 0.3 ^a	0.094
W_2	M	E_1	475.2	62.90 ± 4.6 ^a	0.001	0.2	16.51 ± 0.1 ^a	0.405
		E_2	168.9	44.58 ± 2.5 ^b	0.170	0.2	16.61 ± 0.1 ^a	0.438
		E_3	176.4	36.27 ± 5.0 ^b	0.001	0.3	16.44 ± 0.2 ^a	0.775
	F	E_1	281.3	61.78 ± 2.0 ^a	0.000	0.2	16.52 ± 0.1 ^a	0.034
		E_2	110.8	45.09 ± 1.3 ^b	0.097	0.7	16.27 ± 0.1 ^b	0.435
		E_3	63.5	37.36 ± 3.6 ^b	0.001	0.5	16.55 ± 0.3 ^{a,b}	0.938
W_3	E_1	861.9	84.87 ± 11.1 ^a	0.272	0.4	17.77 ± 0.2 ^a	0.257	
	E_2	146.9	71.29 ± 2.6 ^a	0.000	0.5	17.44 ± 0.1 ^{a,b}	0.091	
	E_3	148.9	53.77 ± 1.5 ^b	0.032	0.3	17.16 ± 0.1 ^b	0.042	

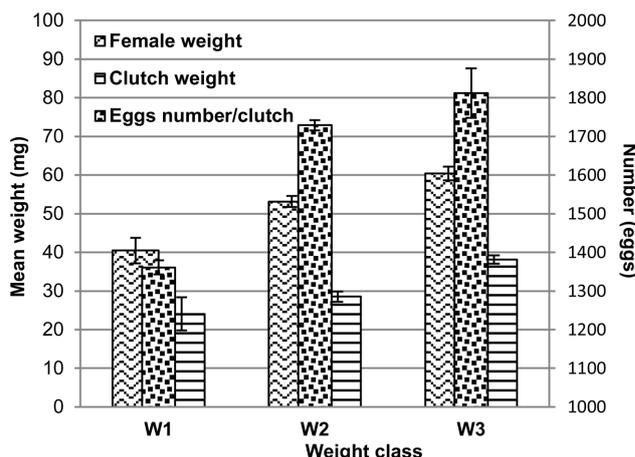


Fig. 7. Mean female and clutch weights, and number of eggs per clutch recorded for the different weight classes.

$df = 2$; $P = 0.086$), were similar. For W_3 individuals there were few males ($N = 8$) and the statistical analysis was not performed; however, the weight loss of females was significant ($F = 24.17$; $df = 2$; $P = 0.000$). In terms of the mean length of the individuals that died early (E_1) and late (E_3) the latter were significantly shorter ($F = 4.28$; $df = 2$; $P = 0.017$) (Table 2).

There was no significant correlation between mean body weight and mean length for males ($p > 0.05$), but a distinctly significant correlation ($r = 0.514$; $p < 0.01$) for W_1 females. In addition, a very significant correlation ($r = 0.517$; $p < 0.001$) was recorded for W_3 females.

Among the males in the different weight classes there are significant differences in body weight ($F = 15.00$; $df = 2$; $P = 0.000$) and length ($F = 38.47$; $df = 2$; $P = 0.000$), with W_3 individuals heavier than those of W_2 and W_1 (Table 3).

Among the females in the different weight classes there were significant differences in body weight ($F = 16.58$; $df = 2$; $P = 0.000$) and length ($F = 103.03$; $df = 2$; $P = 0.000$), with W_3 individuals heavier and longer than those of W_2 and W_1 (Table 3).

Among the weight classes, the same significant differences were recorded for both mean body weight ($F = 38.43$; $df = 2$; $P = 0.000$) and mean length ($F = 150.26$; $df = 2$; $P = 0.000$) of adults of *H. illucens*.

Table 3. Differences in mean body weight and length of adults that emerge from the different weight class treatments. Different letters in a column indicate significant inter-group differences depending on sex, at a 5% level using t-tests as the post hoc test. S^2 – variance.

Sex	Weight class	N	Adult weight (mg)			Adult length (mm)		
			S^2	$\bar{X} \pm SEM$ (mg)	P-value	S^2	$\bar{X} \pm SEM$ (mm)	P-value
M	W_1	56	156.3	39.1 ± 1.7 ^a	0.000	0.5	15.78 ± 0.1 ^a	0.000
	W_2	57	381.7	50.6 ± 2.6 ^b	0.016	0.2	16.55 ± 0.1 ^b	0.021
	W_3	8	265.5	69.0 ± 5.8 ^c	0.001	0.5	17.28 ± 0.2 ^c	0.000
F	W_1	31	327.8	40.46 ± 3.3 ^a	0.001	0.3	15.45 ± 0.1 ^a	0.000
	W_2	138	269.4	53.16 ± 1.4 ^b	0.002	0.5	16.40 ± 0.1 ^b	0.000
	W_3	91	298.4	60.40 ± 1.8 ^c	0.000	0.4	17.27 ± 0.1 ^c	0.000
M&F	W_1	87	214.7	39.59 ± 1.6 ^a	0.000	0.4	15.66 ± 0.1 ^a	0.000
	W_2	195	301.8	52.42 ± 1.2 ^b	0.000	0.4	16.45 ± 0.1 ^b	0.000
	W_3	99	298.6	61.10 ± 1.7 ^c	0.000	0.4	17.27 ± 0.1 ^c	0.000

Table 4. Egg and clutch weight, and numbers of eggs per clutch recorded for the different weight classes. *Different letters in a column indicate significant inter-group differences at a 5% level using t-tests as the post hoc test for individual egg weight. * $p < 0.01$ – distinct differences (Mann-Whitney test).

Weight classes	$\bar{X} \pm \text{SEM}$ (mg) (imago ♀)	Clutch (N)	Egg clutch weight		Egg number/clutch		Egg weight	
			$\bar{X} \pm \text{SEM}$ (mg)	Mann-Whitney test	$\bar{X} \pm \text{SEM}$ (eggs no.)	Mann-Whitney test	$\bar{X} \pm \text{SEM}$ (mg)	T-test P-value
W ₁	40.46 ± 3.3	7	24.07 ± 4.3	$p < 0.01$	1361 ± 18	$p < 0.01$	0.017 ± 0.0 ^a	0.917
W ₂	53.16 ± 1.4	10	28.53 ± 1.3	$p < 0.01$	1729 ± 13	$p < 0.01$	0.017 ± 0.0 ^{a,b}	0.005
W ₃	60.40 ± 1.8	20	38.12 ± 1.1	$p < 0.01$	1812 ± 64	$p < 0.01$	0.021 ± 0.0 ^c	0.035

There were significant differences ($F = 14.97$; $df = 1$; $P = 0.000$) in the female body weight (54.18 ± 1.1 mg) and male body weight (46.51 ± 1.7 mg), in the entire population. Comparison of the differences of the body length of females (16.59 ± 0.1 mm) with that of males (16.24 ± 0.1 mm) revealed significant differences ($F = 14.93$; $df = 1$; $P = 0.000$), which provide statistical support for sexual dimorphism (Fig. 5).

There was a higher number of males in the lowest weight class (W₁) and higher number of females in the heavier weight classes (W₂ and W₃) (Fig. 6).

Relationship between female weight and clutch quality parameters

Oviposition started 4 days after adult emergence and first mating was recorded 2 days before oviposition.

There were significant differences ($F = 15.60$; $df = 2$; $P = 0.000$) in the weight of the clutches oviposited by the females in the different weight classes. In addition, there were significant differences between the clutches laid by the different weight classes in terms of mean egg number ($F = 4.10$; $df = 2$; $P = 0.025$) and mean egg weight ($F = 9.79$; $df = 2$; $P = 0.000$) (Table 4).

In this study, the highest egg clutch weight was recorded for W₃ (49.0 mg) and the lowest for W₁ (11.5 mg). The egg clutch with the lowest number of eggs (912) eggs was produced by an W₁ individual. The egg clutch with the highest number of eggs was recorded for W₂, with 2355 eggs. The highest number of clutches with more than 2000 eggs was recorded for W₃ ($n = 5$) and for each weight class there were 3–4 clutches with between 1000–1500 eggs.

There are significant correlations between clutch weight and number of eggs for each weight class, but no correlation between clutch weight and egg weight (Table 5). There are significant differences between mean egg clutch weight and number of eggs, with a positive correlation with mean female body weight for each weight class (Fig. 7.).

Table 5. Correlation coefficients for the relationships between clutch weight and the number and weight of eggs. n.s. – not significant; * – significant; ** – highly significant; *** – very highly significant.

Weight class	Clutch weight and egg number (r)	Clutch weight and egg weight (r)
W ₁	0.942**	0.640 ^{ns}
W ₂	0.789*	-0.242 ^{ns}
W ₃	0.919***	0.026 ^{ns}

DISCUSSION

The weight losses recorded are significant in each stage of the biological cycle of the *H. illucens* and have significant effects on egg clutch weight and numbers of eggs per clutch.

Body weight in the prepupal stage

The prepupal period was 21 days for all weight classes and similar to the 20.1–21.6 days reported by Nguyen et al. (2013). The percentages of prepupae that pupated was 3% for W₁, 98.5% for W₂ and 97% for W₃. The few that did not pupate can be attributed to the handling of pupae while determining their weight. This is confirmed by a previous experiment (unpublished), which involved weighing prepupae daily and resulted in a drastic decrease in the percentage pupation to under 10% and doubling of the length of the prepupal period to 42 days during which over 90% of the prepupae died. Despite this, the results are comparable with those of Park et al. (2010) of 97.5–99.8%, regardless of season. The weight loss recorded for W₁ prepupae was 12.75% of the initial weight, for W₂ prepupae 18.88% and for W₃ 20.56%. Therefore, the weight loss in the prepupal stage increased with increase in the weight class. The mean body weight of the prepupae was 226.0 mg, which is similar to the 212.0–218.0 mg recorded by Lalander et al. (2018) on a similar medium.

Body weight in the pupal stage

The pupation period was similar for W₁ and W₂ (8 days), but for W₃ it was longer (12 days), even though the peak in pupation occurred on day 5 in all three weight classes. The mean length and mean body weight of the individuals at pupation differed significantly between weight classes ($P = 0.000$). The mean weight and length of the individuals that pupated early in W₁ were 11.53% and 5.65% greater, respectively, than that of those that pupated later. For W₂ the mean weight of the individuals that pupated late was greater by 7.25% and mean length by 0.83%, than that of those that pupated early. For W₃ the mean weight of those that pupated early was 2.71% greater than those that pupated late. The length of the individuals that pupated early was 3.86% greater than those that pupated late. For W₁ and W₃ there were significant correlations ($p < 0.001$) between mean pupal weight and length. Therefore, the length of those that pupated late was shorter. The data indicate that heaviest individuals at the end of the pupation period lost less weight than those with lower body weights (3.86% vs 11.53%), whose pupation period was 4 days longer.

The weight losses during the length of pupal period did not differ for the different weight classes. The pupal period was 15 days and similar to that reported by Zhang et al. (2010). The pupal period recorded in this study is also similar to the values reported by Gobbi et al. (2013) of 16 to 19 days, depending on the feeding medium, but longer than the 6.9–8.8 days reported by Holmes et al. (2012).

In the pupal stage weight loss is continuous with the weight of the pupae just prior to adult emergence less than the weight at pupation by 29.51% for W_1 , 15.64% for W_2 and 6.09% for W_3 . Weight loss in the pupal stage was inversely correlated with the weight class. Thus, heaviest individuals had relatively the lowest weight losses over the same period (15 days).

The weight of the exuviae reflects the difference in weight of the prepupae and pupae. Therefore, the mean weight of the exuviae was significantly greater for W_3 than for W_2 and W_1 . Tomberlin et al. (2002) report a mean weight of exuviae ranging from 11.0 to 25.0 mg, values similar to those recorded for W_1 and W_2 , but lower than that for W_3 (45.7 mg).

Body weight in the adult stage

The percentage of flies that emerged was identical for W_1 and W_2 (97.9%) and higher for W_3 (99.0%). This is similar to that reported by Park et al. (2010), who mention a percentage of 97.7–99.9%, which is higher than that of 74.2–96.7% reported by Tomberlin et al. (2009).

The longevity of adult *H. illucens* was 11–15 days for W_1 , which is similar to the 12.3–14.1 days reported by Nakamura et al. (2016). The longevity of W_2 and W_3 adults was shorter, 7–10 d and 8–11 d, but these results are comparable with those presented by Miranda et al. (2019), who mention an adult longevity of 4.7–8.2 days. The values recorded for the three weight classes are within the limits presented by Heussler et al. (2018), which are 4 to 15 days for reproductive adults. That adult longevity increases with increase in body weight (Tomberlin et al., 2009) is not confirmed by our results, because mortality occurred earlier for individuals from the highest weight class. There were differences in the longevity of the sexes, especially the adults of the lowest weight class. The males from W_1 and W_2 lived longer by 3–5 days than the females. For W_3 , probably due to the low number of males (8♂ vs 91♀), the longevity of both sexes was similar. These results confirm those of Nakamura et al. (2016), who report that males live 4–5 days longer than females, and of Tomberlin et al. (2009), who report that males lives 3.5 days longer than females.

Regarding the changes in body weight during the adult stage, for W_1 it is clear that there was a significant loss ($P = 0.000$) between the first and last deaths of 45.9% for males and 55.2% for females (Table 5). For W_2 it was also significant ($P = 0.000$) with 42.3% for males and 39.5% for females. The greater male weight loss can be explained by their living for considerably longer than females. For W_3 it was also significant ($P = 0.000$) with 36.5% for females and 27.7% for males. The weight loss of females can

be explained by their reproductive effort and the energy they invested in the development and oviposition of eggs. There were also significant correlations between weight and length of females for W_1 and W_3 .

The differences between sexes in the different weight classes in body weight, length and weight loss were significant. For W_1 , the mean body weight of males was lower than that of females by 3.3%. For W_2 it was 4.76% and for W_3 the mean body weight of females was 14.3% higher than that of males. In the entire population, the females were 14.2% heavier than the males (Fig. 5). This is close to the values reported by Tomberlin et al. (2009) of females being 17–19% heavier on average than males. Sexual dimorphism is confirmed by the fact that females are significantly heavier and longer than males. Regarding the mean length of the adults, it varied between 15.6–17.7 mm depending on the weight class, which is within the range of 13 to 20 mm reported by Kim et al. (2008) and Oliveira et al. (2015). The mean male length of 16.2 mm is slightly longer than the maximum of 15.5–16.0 mm reported by Gobbi et al. (2013), but the mean female length of 16.6 mm is within the 15.6–16.8 mm reported by the same authors. Further to Oliveira et al. (2015), who state that only the genitalia are sexually dimorphic, we can add that the sexes also differ significantly in terms of their length.

The sex ratio for W_1 was 64.4% males and 35.6% females (1.8♂ : 1♀). For W_2 the males made up 29.2% and females 70.8% (0.4♂ : 1♀). For W_3 it was more favourable to females, with 91.9% and only 8.1% males (0.1♂ : 1♀). Tomberlin et al. (2002) report a female percentage of 55.2–60.5%. Gobbi et al. (2013) report 54–62% females in a population reared on 3 types of feed. In the entire population studied there was 70.8% females and 29.2% males.

Relationships between female weight and clutch quality parameters

For W_1 the females had laid 71% of their egg clutches within 4 days of emerging, while for W_2 and W_3 they had laid 70% and 75%, respectively, within 4–5 days of emerging. This pre-oviposition period is similar to the 4.4–4.6 days reported by Nakamura et al. (2016) and comparable with the 2–4 days reported by Heussler et al. (2018). In addition, Tomberlin & Sheppard (2002) using similar rearing conditions report 72.9% having laid eggs 4 days after emergence. Recently, Bertinetti et al. (2019) report that oviposition did not start earlier than 4 days after emergence in any of their treatments.

The oviposition period was 4 days for W_1 and 5 days for W_2 and W_3 . According to Bertinetti et al. (2019) the maximum daily egg production occurs on the fifth day after emergence, which is comparable with the results presented. Nakamura et al. (2016) report a longer oviposition period of 7.6–9.4 days, with the peak in oviposition on the 6th and 7th day after adult emergence, depending on the type of light source. Moreover, Heussler et al. (2018) report an oviposition period of 8 to 13 days.

Among the three weight classes there were significant differences ($p < 0.01$) in the mean egg clutch weights re-

corded. The value for W_3 (38.12 mg) is higher by 25.2% and 36.9%, respectively, than that for W_2 (28.53 mg) and W_1 (24.07 mg). The mean clutch weight for the entire population is 30.24 mg, which is close to the 29.1 ± 1.9 mg reported by Booth & Sheppard (1984). Bertinetti et al. (2019) report a mean clutch weight of 27.1 mg, which is close to the general mean recorded in the present study. Tomberlin et al. (2002) report a mean clutch weight of 14.5–15.9 mg. As there was an increase in the mean clutch weight with increase in female body weight, the highest clutch mean weight was recorded for W_3 , the females of which are the heaviest and the lowest mean clutch weight was recorded for W_1 , the females of which were the lightest. Intermediate mean clutch and female body weights were recorded for W_2 (Fig. 6).

The clutch weight is directly associated with the number of eggs in each clutch and the weight of the female. The lowest mean number of eggs was recorded for W_1 and the highest for W_3 . Therefore, the egg clutches for W_3 contained 1812 ± 64 eggs, which is 24.9% ($p < 0.01$) more than that recorded for W_1 (1361 ± 184) and 4.6% ($p < 0.01$) more than for W_2 (1729 ± 126). Some sources cite a mean number of eggs/clutch lower than recorded in this study, with 320–620 eggs reported by Tomberlin & Sheppard (2002); 603–689 by Tomberlin et al. (2002); 240–289 by Nakamura et al. (2016); 620–700 by Barros et al. (2018) and 412–478 by Bertinetti et al. (2019). Kim et al. (2008) report a mean number of 1001 ± 247 eggs per female, a value close to the 1361 ± 184 eggs recorded for W_1 . Bertinetti et al. (2019) report an egg clutch with 1060 eggs, but also one with only 70 eggs. Booth & Sheppard (1984) report 546–1505 eggs/clutch.

The very significant correlation between clutch weight and egg number ($r = 0.78–0.94$) indicate that clutch weight is a possible indicator of egg number. Tomberlin et al. (2002) also report the existence of a correlation between clutch weight and the number of eggs ($r = 0.88–0.93$).

There were no differences between W_1 and W_2 in terms of the weight of individual eggs (0.017 mg), but for W_3 it was 19.05% greater. There was no correlation between clutch weight and that of one egg. Thus, egg weight might not affect clutch weight. Kim et al. (2008) report an egg weight of 0.024 mg and Bertinetti et al. (2019) a weight of 0.025 mg, both of which are greater than the weights recorded in this study.

In conclusion, it is clear that the heaviest *H. illucens* have the best clutch quality. The highest mean weight of egg clutches (38.12 mg), mean number of eggs/clutch (1812 eggs) and mean egg weight (0.021 mg) were recorded for W_3 , the individuals of which had the highest mean body weight. The various features of the flies increased with mean body weight of the females. The positive correlation between clutch weight and number of eggs indicates a strong connection between these two traits. The marked occurrence of males in the lowest and females in the highest weight classes gives statistically support to a dimorphism in terms of body weight and length.

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